Phytochemical Analysis
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A Survey of Sesamin and Composition of Tocopherol Variability from Seeds of Eleven Diverse Sesame (*Sesamum indicum* L.) Genotypes using HPLC-PAD-ECD

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Abstract: The objective of this study was to determine the composition and content of sesamin and desmethyl tocopherols such as α-tocopherol (αT), δ-tocopherol (δT) and γ-tocopherol (γT) in seeds of sesame (*Sesamum indicum* L.) for 11 genotypes conserved in the United States Department of Agriculture (USDA), Agricultural Research Service (ARS) and Plant Genetic Resources Conservation Unit (PGRCU) in Griffin, Georgia, USA. Seed accessions studied were collections from eight countries worldwide, including one landrace from Thailand and two cultivars from Texas, USA. Novel methodologies and analytical techniques described herein consisted of reverse-phase high-performance liquid chromatography (HPLC) connected in series with two detection systems specific for each analyte class. Photodiode array detection was employed for sesamin analysis and electrochemical array detection was used in the determination of tocopherols. A preliminary study was conducted to assess sesamin levels in 2003 and tocopherol levels in 2004 from sesame seed samples conserved at the USDA, ARS and PGRCU. In 2005, sesame seed samples were grown, harvested and evaluated for sesamin as well as tocopherol levels. The overall results (n = 3) showed that sesamin, αT, δT and γT levels were 0.67–6.35 mg/g, 0.034–0.175 μg/g, 0.44–3.05 μg/g and 56.9–99.3 μg/g respectively, indicating that the sesame seed accessions contained higher levels of sesamin and γT compared with αT and δT. Statistical analysis was conducted and significant differences were observed among the 11 different sesame genotypes. This suggests that genetic, environmental and geographical factors influence sesamin and desmethyl tocopherol content. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: HPLC-PAD-ECD; electrochemical detection; sesamin; desmethyl tocopherols; sesame seeds.

INTRODUCTION

Sesame (*Sesamum indicum* L.) originated from Africa (Ram *et al.*, 1990) and is used worldwide for food, cooking oil, health food and sweets. Processed oil from sesame seeds increases the shelf-life and improves the flavor and taste of foods largely due to its antioxidant properties. Sesame oil is used as a solvent in drug-manufacturing processes, as a skin softener and for the production of margarine and soap (Dark, 1998). Sesame is a well-known medicinal plant because of the lignan known as sesamin, which is thought to play an important role in physiological activities (Jeng and Hou, 2005). In addition, sesame contains a diverse group of biologically active compounds such as multiple tocopherol homologues [α-tocopherol (αT), δ-tocopherol, (δT) and γ-tocopherol (γT)], tocotrienols, sesamolinol diglucosides and other health promoting agents (Hemalatha and Ghafoorunissa, 2004; Jeng and Hou, 2005; Crews *et al.*, 2006; Moazzami *et al.*, 2006). Studies have reported that sesamin has anti-carcinogenic, anti-atherosclerotic and neuroprotective bioactivities. For example, sesamin inhibits human lymphoid leukaemia cells by the induction of apoptosis and has a suppressive effect against 7,12-dimethylbenz[a]anthracene-induced rat mammary carcinogenesis (Hirose *et al.*, 1992; Miyahara *et al.*, 2000). The antioxidant properties of sesamin have lowered both serum and liver cholesterol levels by inhibiting absorption and synthesis of cholesterol simultaneously in rats (Hirose *et al.*, 1991), as well as low-density lipoprotein cholesterol (LDL-C), which is a risk factor for atherosclerosis in humans (Hirata *et al.*, 1996). Lastly, it has been shown that sesamin has neuroprotective effects on hypoxia-induced apoptotic-like cell death in cultured cortical cells (Hou *et al.*, 2003).

The tocopherols are lipophilic, phenolic compounds of plant origin and are the major constituents of vitamin E. They are believed to prevent age-related diseases such as heart disease and cancer. Tocopherol vitamers are free radical scavengers that act as lipid-soluble antioxidants by aiding in the protection of polyunsaturated fatty acids from lipid peroxidation, thereby preventing the propagation of free radical-mediated events resulting from oxidative or nitrative stress (Halliwell and Gutteridge, 1989; Buettner, 1993; Brigelius-Flohe

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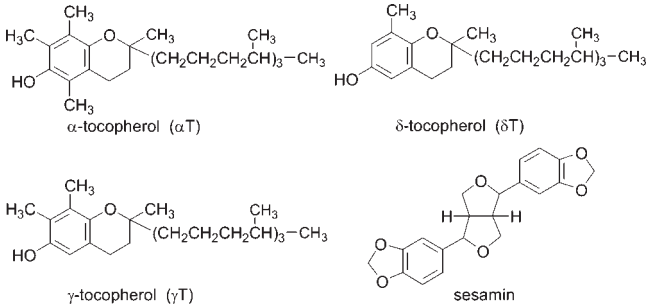

Figure 1 Chemical structures of sesamin, αT, δT and γT, discussed in the text.

and Traber, 1999). A review published by Hensley *et al.* (2004) discussed the classical chemistry and biology of tocopherols with less emphasis on the bioactive properties of αT and greater emphasis on those of γT and its metabolites. These tocopherol analogues are considered to possess anti-inflammatory, anti-neoplastic and natriuretic functions with respect to cancer biology and diseases of the cardiovascular and central nervous system. In fact, consumption of sesame seeds in humans was shown to significantly increase plasma γT (Cooney *et al.*, 2001). In 2005, it was determined that sesamin increased γT in rat plasma as well as in the liver (Frank, 2005). Both sesamin and vitamin E have shown benefits in the prevention of hypertension and stroke (Noguchi *et al.*, 2001, 2004; Tsuruoka *et al.*, 2005).

Since there has been an increase in the use of sesame as a nutraceutical, it is becoming more important to determine the variability in content of these compounds among sesame genotypes. Considering that sesamin and desmethyl tocopherols (αT, δT and γT), depicted in Fig. 1, are responsible for several pharmacological activities ascribed to *S. indicum*, knowledge of the variability in content of sesamin and tocopherols would be beneficial for the identification of superior sesame genotypes, as well as determining possible candidates for use in a breeding programme to enhance sesamin and tocopherol levels. The objective of the present study was to develop innovative methodologies and procedures for the isolation, characterisation and quantitation of sesamin, αT, δT and γT in diverse *S. indicum* genotypes using high-performance liquid chromatography (HPLC) connected in series with two detection systems. A photodiode array detector (PAD) was used to determine sesamin levels, whilst an electrochemical array detector (ECD) was employed in the measurement of tocopherol congeners (αT, δT and γT). This is the first report concerning the levels of sesamin and tocopherol homologues in 11 genotypes conserved in the United States Department of Agriculture (USDA), Agricultural Research Service (ARS) and Plant Genetic Resources Conservation Unit (PGRCU) in Griffin, GA, USA.

EXPERIMENTAL

Reagents and equipment. Unless otherwise stated, all reagents, solvents and equipment used for sesamin/tocopherol protocols were analytical-grade/HPLC-grade or equivalent and purchased from Fisher Scientific (Pittsburgh, PA, USA) or Sigma Chemical Company (St. Louis, MO, USA). The water used for HPLC-PAD-ECD analysis was obtained from a four-stage purification system (Barnstead-Thermolyne, Dubuque, IA, USA) equipped with ion-exchange cartridges to remove dissolved solids and a 0.2 μm polishing filter to remove particulate matter.

Plant materials. All sesame seed accessions are conserved and curated at the USDA, ARS and PGRCU located in Griffin, GA, USA. Seed accessions studied were collections from eight diverse countries, and included one landrace from Thailand, and two cultivars from Texas, USA. Information concerning the origin and plant morphology is provided in Table 1. Seeds

Table 1 Description of sesame genotypes used in study

Accession no.	Name	Origin/collection site	Plant height (dm)	Locule no.	Stem type
PI 163595	1041	Guatemala	15	4	Branched
PI 164387	Til	India	9	4	Not branched
PI 189081	No. 1	Cameroon	18	4	Branched
PI 200111	43-15	Venezuela	18	4, 8	Not branched
PI 247855	21159	Zaire	13	4	Branched
PI 288859	537	Nepal	19	4	Branched
PI 298630	Giza 24	Israel	13	4	Branched
PI 343815	N/A	Iran	11	4	Branched
PI 490026	Landrace	Thailand	6	4	Branched
PI 599436	Llano	Cultivar donated Texas	N/A	4	Branched
PI 599437	Margo	Cultivar donated Texas	6	4	Not branched

N/A: not applicable.

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from 11 sesame accessions were stored at –18°C. The accession numbers are as follows: PI 343815, PI 189081, PI 288859, PI 490026, PI 164387, PI 163595, PI 200111, PI 247855, PI 298630, PI 599436 and PI 599437. Sesame seeds from these stored accessions were submitted to our laboratory to be used in preliminary evaluations for sesamin levels only in 2003. However, it was not until 2004 that αT, δT and γT amounts were determined from the identical sesame seed accessions (listed above). In 2005, sesame seeds regenerated previously from these 11 sesame accessions were used in a second experiment for the assessment of sesamin as carried out previously in 2003, and for desmethyl tocopherols in 2004. The protocol for sesame seed regeneration was as follows: 50–100 fresh seeds from the 11 sesame accessions were planted and grown in field regeneration plots at the USDA, ARS and PGRCU facilities. Sesame plants were hand harvested as they matured (4–5 months after planting), dried at 21°C in 25% relative humidity for 2–4 weeks, and stored at –18°C. The soil type used in this study consisted of a clay-based soil. All sesame plants and seeds were identified by G. Lovell or Dr. J.B. Morris at the USDA, ARS, PGRCU and the University of Georgia.

Seed extraction and sample preparation for sesamin determination. A sample of *ca.* 100–140 mg (dry weight) of each sesame seed lot was comminuted using a tissue homogeniser (Tekmar Company, Cincinnati, OH, USA) and 6 mL of HPLC-grade methanol was added. The mixture was vortex mixed, sonicated (Branson Sonifier Model 250, Branson Ultrasonics Corp., Danbury, CT, USA), and centrifuged at 3000 rpm in an International Equipment Co. (Needham Hts., MA, USA) model HN-SII benchtop centrifuge for 30 min. The supernatant was removed, evaporated to dryness in a stream of high purity nitrogen gas with minimal heating (37°C), and the residue dissolved in 1 mL of HPLC grade methanol. The sesamin extracts were passed through a 0.2 μm, 13 mm PVDF millipore Acrodisk (Fisher Scientific, Norcross, GA, USA) syringe filter and stored at –20°C prior to chromatographic analysis.

HPLC-PAD analysis of sesamin. Characterisation and quantitation of sesamin in the sesame seed lots were achieved by HPLC-PAD using an ESA (Chelmsford, MA, USA) model 582 HPLC solvent delivery system as previously described (Hensley *et al.*, 2000; Williamson *et al.*, 2002, 2003; Hensley and Williamson, 2005) coupled with a Waters (Milford, MA, USA) model 996 PAD detector upstream from a 12-channel ECD detector (ESA model 5600 CoulArray). Isocratic elution of sesamin was carried out using a mobile phase containing acetonitrile:water (50:50, v/v), 30 mM lithium acetate and 50 μL/L of bacteriocide reagent MB (ESA). Analyte separation was conducted on a TosoHaas (Montgomeryville, PA, USA) reverse-phase ODS-TM C_{18} analytical column (250 × 4.6 mm i.d.; 5 μm particle size). The injection volume was 60 μL, the flow rate was 0.50 mL/min, and the detector wavelength was set at 281.7 nm. All chromatograms were collected, recorded and processed on an IBM compatible PC running Waters Empower software.

Seed extraction and sample preparation for tocopherol determination. A sample of *ca.* 100–190 mg (dry weight) of each sesame seed lot was comminuted using a Tekmar tissue homogenizer (Cincinnati, OH, USA), followed by the addition of 2 mL of Millipore filtered water, 1 mL of absolute ethyl alcohol, 13 μL of butylated hydroxytoluene solution (10 mg/mL), and 6 mL of HPLC grade hexane. The sample mixture was vortex mixed, sonicated (Branson Sonifier), and centrifuged at 3000 rpm in an International Equipment Co., model HN-SII benchtop centrifuge for 30 min. The supernatant was removed, evaporated to dryness under a stream of high-purity nitrogen gas with minimal heating (37°C), and the tocopherol homologues were reconstituted in 1 mL of HPLC grade methanol. The tocopherol extracts were passed through a Fisher Scientific 0.2 μm, 13 mm PVDF millipore Acrodisk syringe filter (Norcross, GA, USA) and stored at –20°C prior to HPLC-ECD analysis.

HPLC-ECD analysis of tocopherol homologues. Analysis of tocopherol variants was carried out on an ESA model 582 HPLC solvent delivery system coupled with a 12-channel ECD detector (ESA model 5600 CoulArray) downstream from a Waters model 996 PAD detector (Hensley *et al.*, 2000; Williamson *et al.*, 2002, 2003; Hensley and Williamson, 2005). For isocratic separations, the mobile phase consisted of 83% acetonitrile, 12% methanol, 0.2% acetic acid, 30 mM lithium acetate and 50 μL/L of bacteriocide reagent MB (ESA). Analyte separation was conducted on a TosoHaas reverse-phase ODS-TM C_{18} analytical column (250 × 4.6 mm i.d.; 5 μm particle size). The injection volume was 60 μL, the flow rate was 2 mL/min, and the chromatographic data were collected, recorded and processed on an IBM-compatible PC running ESA CoulArray software.

Data and statistical analysis. In order to statistically assess all three years (2003–2005) of data for each sesame seed accession, two-way analysis of variance (ANOVA) using the Statistical Analysis System (SAS) 2006 software program (SAS Institute, Cary, NC, USA) was employed. Fisher's protected LSD (least significant difference) test was used to separate the means. The data reported was expressed as mean ± standard deviation (n = 3, including preliminary data) and ranked by descending sesamin levels (highest-lowest). In order to characterise variability among these sesame accessions harvested from one environment, to determine correlations, as well as to establish relationships among sesame

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accessions analyzed for chemical components (sesamin, δT, γT and αT), principal component (PC) analysis was used. Standard errors (SE), as well as coefficients of variation (CV) were also determined to confirm variability using principal component analysis (2006 SAS software program, SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Investigational studies assessing sesamin and desmethyl tocopherols (δT, γT and αT) content employing different extraction and analytical methodologies in 11 different genotypic varieties and origins of sesame seeds worldwide are scarce and, as far as we know, very few studies have been published. However, cited manuscripts determining the sesamin and tocopherol composition and content in other grain and oil sources consisting of olive, hazelnut and sesame seed oils, walnuts and maize kernels have been published (Tadmor *et al.*, 2000; Hemalatha and Ghafoorunissa, 2004; Amaral *et al.*, 2005; Crews *et al.*, 2005, 2006; Cunha *et al.*, 2006). More importantly, instrumental techniques used in the characterisation and quantitation of sesamin and tocopherol vitamers with respect to sesame seed cultivars have included reverse-phase HPLC (RP-HPLC) and normal-phase HPLC (NP-HPLC) technologies in conjunction with ultraviolet and photodiode array spectrophotometric detection systems (Hemalatha and Ghafoorunissa, 2004; Crews *et al.*, 2006).

Unfortunately, the use of RP-HPLC-ECD has not been documented in the literature for this particular application. Therefore, additional analytical detection systems consisting of HPLC-PAD-ECD (as demonstrated in this publication), programmable fluorescence detection (HPLC-PFD), evaporative light scattering detection (HPLC-ELSD), HPLC or gas chromatography (GC) coupled with mass spectrometry (HPLC-MS/GC-MS) could be implemented provided adequate sensitivity, selectivity, method optimisation and validation parameters are used to ensure consistent and accurate results for all analytes of interest.

Characterisation, identification and quantification of sesamin

HPLC-PAD chromatographic analysis of sesamin were characterised and identified by comparing spectrophotometric properties, retention times and peak heights of sesame seed samples with those of serial dilutions of authentic sesamin standards at varying concentration levels ranging from 1 to 500 μg/mL in order to generate a series of calibration curves and demonstrate instrument efficiency. External calibration standards were prepared fresh from a stock sesamin standard prior to each HPLC-PAD analysis set, interspersed regularly among the samples during automated analyses, and the stock sesamin standard was stored at –20°C to ensure analyte stability and accuracy. The 11 extracted sesame seed genotypes containing sesamin were quantified by measuring the peak heights of the sample extracts relative to the peak heights of the standard sesamin calibration curves previously generated. The minimum quantifiable limit (MQL) of sesamin by HPLC-PAD was 60 ng (injected on column). Elution profiles for a 100 μg/mL sesamin standard and PI 490026 sesame seed sample depicting the two-dimensional (2D) contour plots, ultraviolet–visible (UV–vis) spectrum and retention times (between 29 and 32 min) are illustrated in Figs 2 and 3.

Comparison of the 2D contour plots and UV–vis spectra used in the HPLC-PAD analysis provide a positive "fingerprint match" for the characterisation in sesamin standards and sesamin content in sesame seed accession samples. The combination of parameters consisting of wavelength, absorbance and retention time(s) generated from these plots (Fig. 3) aid in the identification and quantitation of sesamin. Exact retention times may vary somewhat depending on column, temperature and mobile phase composition. Linearity of sesamin was established and the regression coefficients were greater than 0.99. Based on the above HPLC-PAD analysis, sesame seed sample lots were quantitated, converted and reported for sesamin content in Tables 2 and 3 (mg/g). Representative HPLC-PAD chromatograms of the sesame accessions containing sesamin are depicted in Figs 2 and 4.

Characterisation, identification and quantification of desmethyl tocopherols

In order to aid in the characterisation and identification of δT, γT and αT by HPLC-ECD, hydrodynamic voltammograms were generated using authentic tocopherol standards for comparative purposes to determine the assignment of chromatographic peaks in both the sesame seed samples and tocopherol standards, as well as determining the optimal oxidation potential for each analyte based on their electrochemical properties, peak heights, and retention times. Figure 5 compares the different hydrodynamic voltammograms of an authentic 10 μM tocopherol standard mixture containing δT, γT and αT. Oxidation potentials employed for the measurement of tocopherols under these conditions are listed in Table 4. The optimal oxidation potential for each tocopherol congener was 400 mV.

Comprehensive reviews concerning the theory, principles and hydrodynamic voltammogram generation by ECD detection have been published elsewhere (Hensley *et al.*, 1999, 2000; Hensley and Williamson, 2005); therefore, discussion of these details is omitted. Quantification of tocopherol analytes in 11 sesame genotypes

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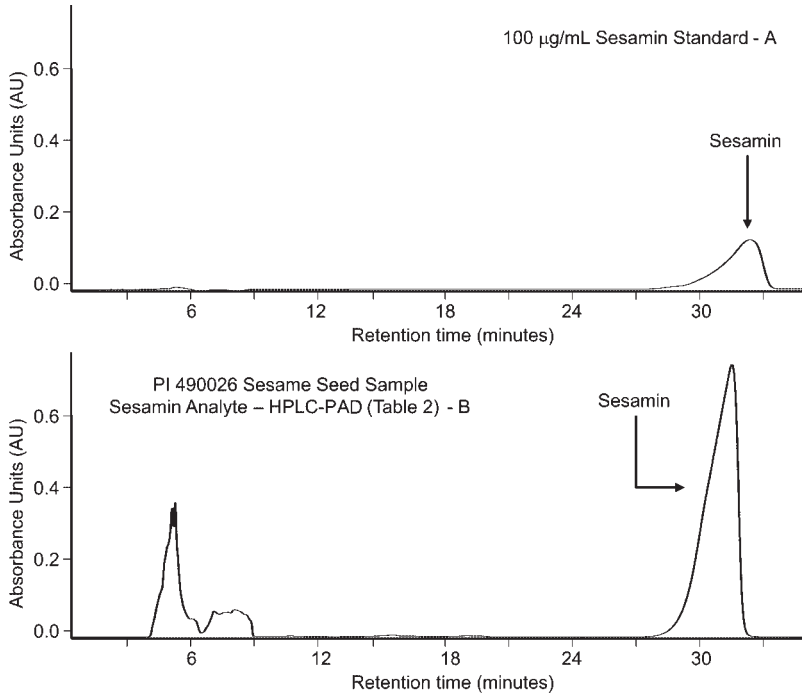

Figure 2 HPLC-PAD chromatograms of an authentic 100 μg/mL sesamin standard (A) and (B) an extracted *S. indicum* sesame seed sample containing sesamin, accession no. PI 490026 (Table 2), obtained following the procedures and methodologies described in the Experimental section.

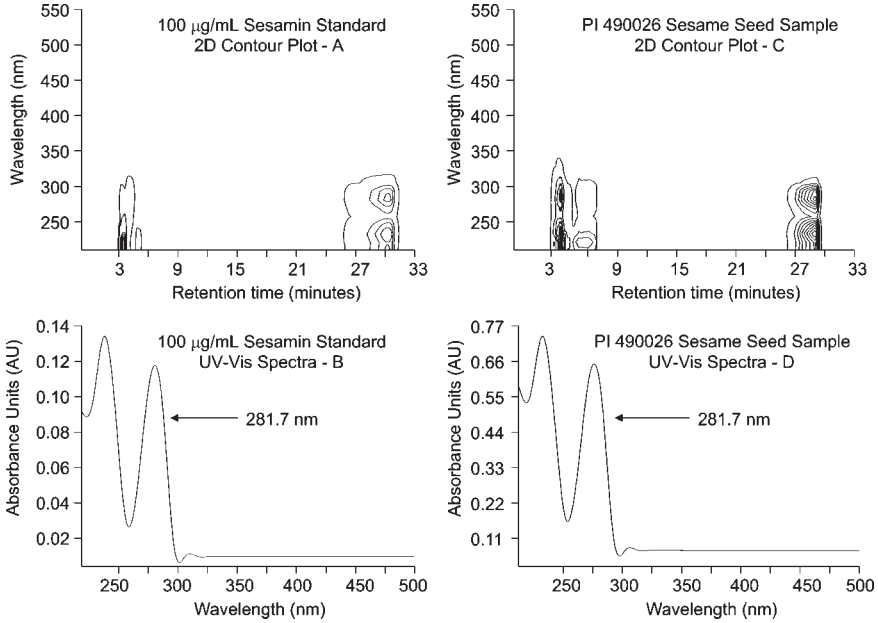

Figure 3 HPLC-PAD detector output illustrating two-dimensional contour plots and UV–vis specta of the 100 μg/mL sesamin standard (A, B) and *S. indicum* sesame seed sample containing sesamin, accession no. PI 490026 (Table 2) (C, D), shown in Fig. 2. The optimal PAD wavelength for sesamin was 281.7 nm.

were carried out by performing serial dilutions using a standard mixture containing δT, γT and αT prepared at varying concentration levels ranging from 0.050 to 10 μM in order to generate a series of calibration curves and demonstrate instrument efficiency. External calibration standards were prepared fresh from stock tocopherol standards prior to each HPLC-ECD analysis set, interspersed regularly among the samples during automated analyses, and the stock desmethyl tocopherol standards were stored at –20°C to ensure analyte stability and accuracy. The extracted sesame seed sample cultivars were quantified by measuring the

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Table 2 Seed colour, seed weight, and sesamin content in 2003,[a] and α-tocopherol, δ-tocopherol and γ-tocopherol content in 2004[a] from 11 sesame genotypes ranked by sesamin (preliminary study)

Accession no.	Seed colour	Seed weight (mg)[b]	Sesamin (mg/g) PAD	Seed weight (mg)[c]	Tocopherol (μg/g) ECD		
					αT	δT	γT
PI 247855	Variable	120	5.97	180	0.064	1.95	85.0
PI 163595	White	100	5.73	150	0.056	3.62	82.2
PI 298630	Tan	130	5.36	100	0.127	0.54	70.4
PI 200111	Yellow	110	5.00	130	0.068	1.81	84.6
PI 599436	Brown	140	4.77	190	0.067	2.05	63.4
PI 599437	Tan	100	4.33	170	0.351	2.34	81.6
PI 343815	Black	100	3.02	170	0.198	1.66	77.0
PI 490026	Black	110	2.96	170	0.034	1.03	65.7
PI 288859	Buff	110	2.00	120	0.031	0.89	69.5
PI 189081	Tan	130	1.77	140	0.082	1.88	78.9
PI 164387	Brown	130	0.83	120	0.066	2.33	97.2
Mean		116	3.79	148	0.104	1.83	77.8
SD[d]		14.3	1.77	26.5	0.093	0.840	9.9

[a]n = 1. [b]Seed weights for sesamin analysis. [c]Seed weights for tocopherol analysis. [d]SD: Standard deviation.

Table 3 Mean seed weight, sesamin, α-tocopherol, δ-tocopherol and γ-tocopherol content from seeds of 11 accessions of sesame, ranked by sesamin in 2003–2005[a]

Accession no.	Seed weight (mg)[b]	Sesamin (mg/g) PAD	Seed weight (mg)[c]	Tocopherol (μg/g) ECD		
				αT	δT	γT
PI 247855	106.6ab	6.35a	110.0a	0.051ab	2.26b	97.2ab
PI 298630	113.3a	6.14a	106.6a	0.150ab	0.441f	56.9e
PI 599436	80.0b	5.62ab	113.3a	0.124ab	1.69cd	67.4de
PI 163595	113.3a	5.26bc	123.3a	0.037b	3.05a	80.5bcd
PI 599437	103.3ab	4.52c	126.6a	0.175a	1.85bc	70.9cde
PI 200111	116.6a	4.45c	113.3a	0.050ab	1.67cd	87.5abc
PI 490026	110.0ab	3.17d	133.3a	0.051ab	1.22de	68.1de
PI 343815	103.3ab	3.16d	126.6a	0.123ab	1.62cd	76.5cd
PI 189081	110.0ab	2.43de	86.6a	0.067ab	1.84bc	99.3a
PI 288859	116.6a	2.14e	126.6a	0.076ab	1.06e	77.5cd
PI 164387	120.0a	0.67f	120.0a	0.034b	2.28b	97.2ab
Mean	108.5	3.99	116.9	0.085	1.72	79.9
SD[d]	18.5	1.81	34.3	0.078	0.71	16.0

[a]Values are means (n = 3). [b]Seed weights for sesamin analysis. [c]Seed weights for tocopherol analysis. [d]SD: Standard deviation. ANOVA for seed weight (sesamin analysis), sesamin, αT, δT and γT showed significant differences within parameters ($p < 0.05$); means within a column followed by the same letter indicate no difference, Fisher's LSD test ($p < 0.05$).

peak heights of the sample extracts relative to the peak heights of the standard calibration curves previously generated (400 mV oxidation potential).

The minimum quantifiable limit (MQL) of tocopherol homologues by HPLC-ECD was 3 pmoles (on column). A characteristic elution profile of a 10 μM tocopherol standard mixture (δT, γT and αT) illustrating their retention times was determined to be 13, 17 and 21 min, respectively, as depicted in Fig. 6. However, exact retention times may vary somewhat depending on column, temperature and mobile phase composition. Linearity of each tocopherol variant was established and the regression coefficients were greater than 0.99. HPLC-ECD analysis δT, γT and αT levels from sesame seed sample lots were determined, converted and reported in Tables 2 and 3 (μg/g). Representative HPLC-ECD chromatograms of the sesame accessions containing the desmethyl tocopherols are depicted in Figs 6 and 7.

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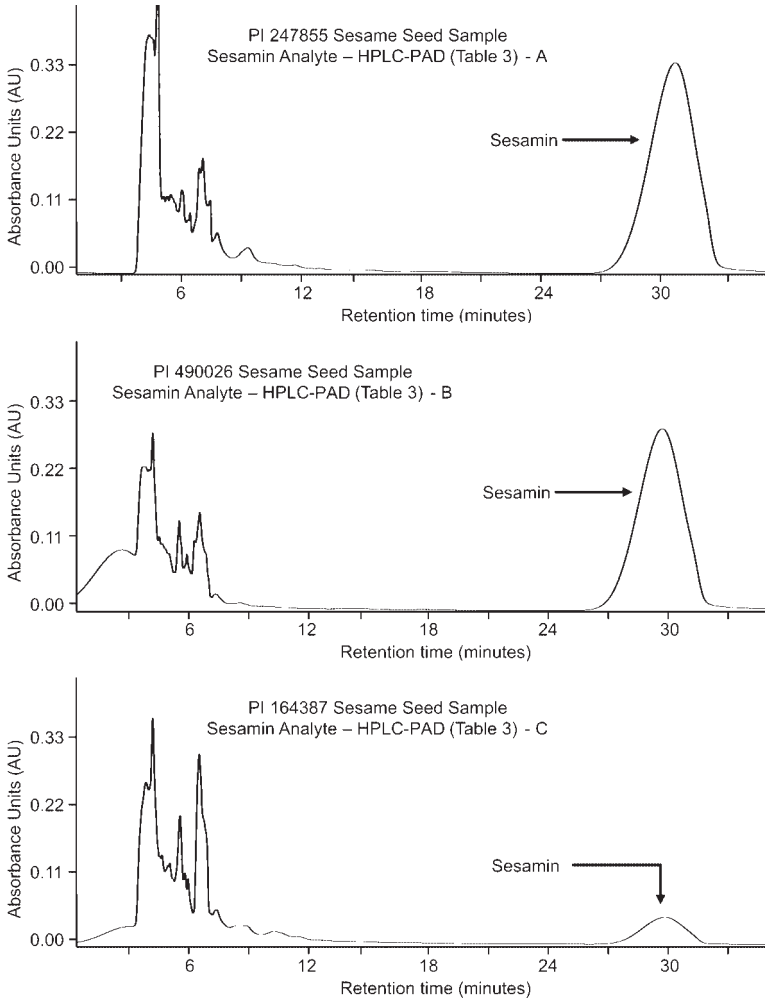

Figure 4 (A–C) HPLC-PAD chromatograms of extracted and processed *S. indicum* sesame seed samples containing sesamin. Accession numbers: (A) PI 247855, (B) PI 490026 and (C) PI 164387 (Table 3) represent the specific genotype ranked by descending sesamin levels (highest to lowest).

Table 4 Selected cell potentials for electrochemical array detection of tocopherol congeners using a 12-channel instrument

Cell number	Cell potential (mV)	Cell number	Cell potential (mV)
1	200	7	650
2	300	8	675
3	400[a]	9	700
4	525	10	750
5	600	11	825
6	625	12	900

[a]Optimal cell potential for tocopherol analytes.

Content of sesamin in 2003, α, δ and γ-tocopherol in 2004 from seeds of 11 *S. indicum* genotypes

Individual sesame seed accessions originating from the USDA, ARS and PGRCU repository (stored at −18°C) were employed in 2003 and 2004 for a preliminary study to assess sesamin (HPLC-PAD) and tocopherol congener levels (HPLC-ECD). This preliminary survey was done only to assess the content of these analytes using individual samples (n = 1) in order to determine variability among sesame genotypes. Illustrated below are the findings of this investigational survey. The results are shown in Table 2. All data are expressed as mean ± standard deviation (n = 1) and ranked by descending sesamin levels (highest–lowest).

Sesamin

Sesamin content ranged from 0.83 to 5.97 mg/g among the 11 *S. indicum* genotypes in 2003 (Table 2). The accessions with the largest amounts of sesamin were PI 247855 (5.97 mg/g), PI 163595 (5.73 mg/g), PI 298630 (5.36 mg/g) and PI 200111 (5.00 mg/g). The next highest amounts of sesamin were found

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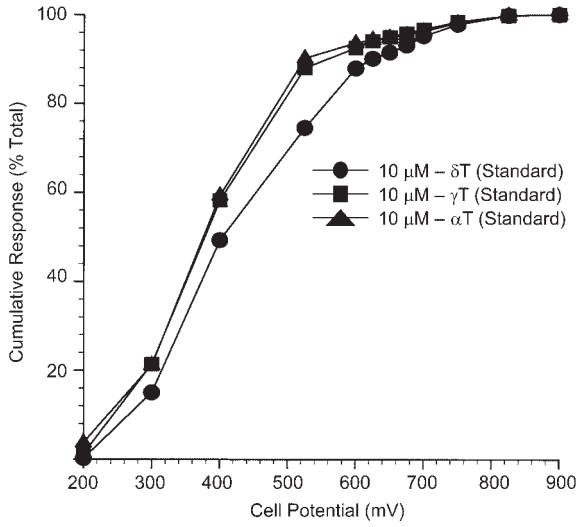

Figure 5 Hydrodynamic voltammograms of 10 μM δT, 10 μM γT and 10 μM αT standards. The plots generated are a fractional percentage of the cumulative current response (percentage total) vs the cell potential (mV). The data collected from the chromatographic run to generate the voltammograms was done using a 12-channel coulometric array detector (HPLC-ECD), as discussed in the text. The optimal oxidation potential for each tocopherol congener was determined to be 400 mV.

in PI 599436 and PI 599437, averaging 4.55 mg/g of sesamin, while the lowest amounts were observed in PI 343815, PI 490026, PI 288859, PI 189081 and PI 164387, averaging 2.11 mg/g of sesamin.

α-Tocopherol

The amount of αT ranged from 0.031 to 0.351 μg/g among the 11 *S. indicum* genotypes in 2004 (Table 2). The accessions with the largest amounts of αT were PI 599437 (0.351 μg/g), PI 343815 (0.198 μg/g) and PI 298630 (0.127 μg/g). The next largest amounts of αT occurred in PI 189081, PI 200111, PI 599436, PI 164387, PI 247855 and PI 163595, averaging 0.067 μg/g of αT. The lowest amount of αT occurred in both PI 490026 and PI 288859, averaging 0.032 μg/g.

δ-Tocopherol

The level of δT ranged from 0.54 to 3.62 μg/g among the 11 *S. indicum* genotypes in 2004 (Table 2). Accessions producing the most δT were PI 163595 (3.62 μg/g), PI 599437 (2.34 μg/g), PI 164387 (2.33 μg/g) and PI 599436 (2.05 μg/g). Next in order of δT production were PI 247855, PI 189081, PI 200111, PI 343815 and PI 490026, averaging 1.66 μg/g. The lowest amount of δT was found in both PI 288859 and PI 298630, averaging 0.71 μg/g.

γ-Tocopherol

All 11 *S. indicum* genotypes in 2004 produced a very large amount of γT with contents ranging from 63.4 to

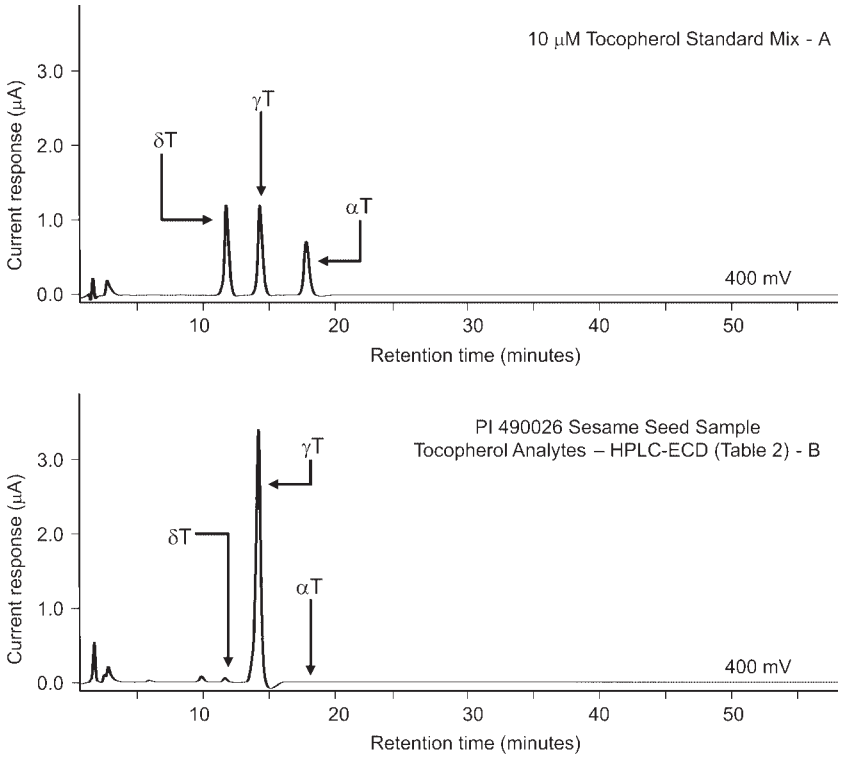

Figure 6 HPLC-ECD chromatograms of an authentic 10 μM tocopherol mix standard containing αT, δT and γT variants (A), and (B) an extracted *S. indicum* sesame seed sample containing the tocopherol analytes (αT, δT and γT), accession no. PI 490026 (Table 2), obtained following the procedures and methodologies described in the Experimental section.

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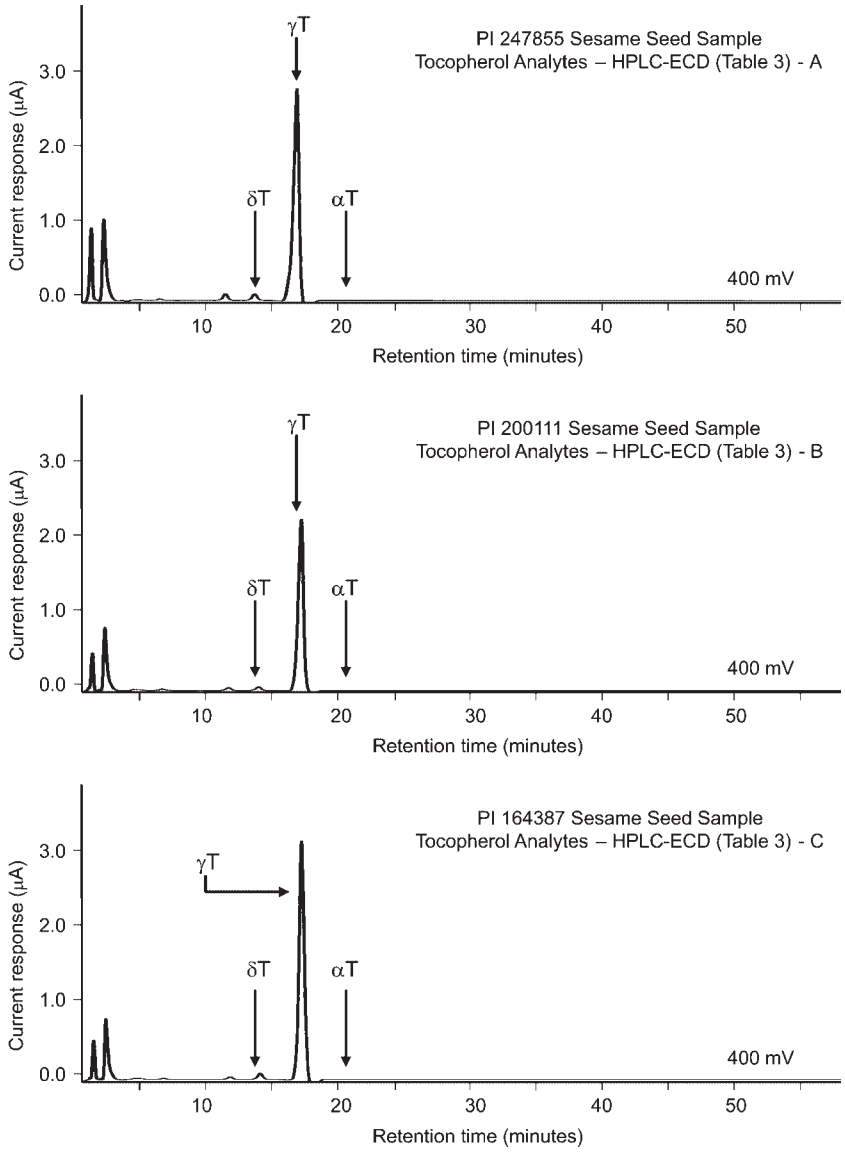

Figure 7 (A–C) HPLC-ECD chromatograms of extracted and processed *S. indicum* sesame seed samples containing desmethyl tocopherols. Accession numbers: (A) PI 247855, (B) PI 200111 and (C) PI 164387 (Table 3) represent the specific genotype ranked by descending sesamin levels (highest to lowest).

97.2 μg/g (Table 2). The greatest amount of γT was found in PI 164387 (97.2 μg/g), PI 247855 (85.0 μg/g), PI 200111 (84.6 μg/g), PI 163595 (82.2 μg/g) and PI 599437 (81.6 μg/g). Next in order of γT production were PI 189081, PI 343815 and PI 298630, averaging 75.4 μg/g. The lowest amount of γT production was found in PI 288859, PI 490026 and PI 599436, averaging 66.2 μg/g.

Content of sesamin, α, δ and γ-tocopherol in seeds of 11 *S. indicum* genotypes in 2005

In 2005, duplicate sesame seed accession samples were grown and harvested from field regeneration plots (USDA, ARS and PGRCU facilities), collected and analysed for sesamin (HPLC-PAD) and tocopherol concentrations (HPLC-ECD) ($n = 2$). However, in order to determine statistical relevance with respect to the data accumulated from the sesame seed cultivars in 2003–2005, ANOVA using the SAS 2006 software program was carried out to analyse all data collected (seed weight, sesamin, αT, δT and γT) and reported as mean values ($n = 3$). Differences between the groups were determined and a p-value less than 0.05 was defined as statistically significant. Fisher's protected LSD test was also used to separate the means. Mean values (as shown in Table 3) in the same column with the same letter are not significantly different, whereas different letters within the same column indicate statistical significance from each other according to Fisher's protected LSD ($p < 0.05$; $n = 3$). Therefore, significant

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Table 5 Eigenvalues and the proportion of total variability among sesame accessions (2003–2005) as explained by the principal components

Principle component	Eigenvalue		% Variability		% Cumulative	
	2003/2004	2005	2003/2004	2005	2003/2004	2005
1	1.5905	1.7999	39.76	45.00	39.76	45.00
2	1.1282	1.0339	28.21	25.85	67.97	70.85
3	0.9169	0.8041	22.92	20.10	90.89	90.95
4	0.3642	0.3620	9.11	9.05	100.00	100.00

differences were observed and the summarised data is depicted below.

Sesamin

The accessions, PI 247855, PI 298630 and PI 599436 produced significantly more sesamin (6.35, 6.14 and 5.62 mg/g, respectively) than most of the other sesame genotypes in 2005 (Table 3). Sesamin levels in PI 163595 (5.26 mg/g), PI 599437 (4.52 mg/g) and PI 200111 (4.45 mg/g) were significantly higher than in the remaining five accessions (0.67– 3.17 mg/g sesamin). These reported values for sesamin levels are in close agreement with the data reported by Hemalatha and Ghafoorunissa (2004).

α-Tocopherol

Only PI 599437 (0.175 μg/g) was significantly higher in αT content than PI 164387 (0.034 μg/g) and PI 163595 (0.037 μg/g) (Table 3). All other αT levels were determined not to be statistically significant based on Fisher's protected LSD test.

δ-Tocopherol

Accessions consisting of PI 163595 (3.05 μg/g), PI 164387 (2.28 μg/g) and PI 247855 (2.26 μg/g) were significantly higher in δT content than most of the sesame genotypes (Table 3). The accession, PI 298630 (0.44 μg/g) produced significantly lower δT than all other sesame genotypes. The remaining seven accessions ranged from 1.06 to 1.85 μg/g of δT .

γ-Tocopherol

The accession, PI 189081 (99.3 μg/g) produced significantly higher γT than all other sesame accessions and PI 298630 (56.9 μg/g) produced significantly less γT than most of the other accessions (Table 3). Also, the remaining nine accessions varied from 67.4 μg/g to 97.2 μg/g of γT. Overall, significant variable amounts as well as similar rankings were observed with respect to these 11 sesame genotypes for sesamin, αT, δT and γT in 2005 compared with the analysis conducted in 2003 and 2004 (Table 3).

Principal component analysis

The first, second and third principal components (PC) collectively accounted for 90.9% and 91.0% of the total variability in sesame accessions, respectively shown in Table 5. Thus, sample variation can be summarised by the first three principal components for sesame accessions during 2003–2005, illustrated in Table 6. Correlation coefficients between the first three components and the biochemical data set (sesamin, αT, δT and γT) were determined in order to ascertain the relative importance of each sesamin and tocopherol analyte in explaining the variation within the first three principal components (Table 6). We observed that the biochemical data in the first principal component was primarily correlated with δT and γT. On the basis of these coefficients, the first principal component appears to contrast δT and γT with αT, as well as sesamin. The second principal component was strongly correlated with sesamin and appears to contrast sesamin with αT, δT and γT.

Lastly, the third principal component was strongly correlated with αT and appears to contrast αT with sesamin, δT and γT. Since αT (0.87 and 0.83 PC3) and sesamin (0.83 and 0.74 PC2) represent the largest fraction, their contribution to sample variation is greater than the contributions by δT or γT. The coefficients of variation for αT and sesamin (2003–2005) were 90, 91, 46 and 45% in sesame accessions, respectively. Coefficients of variation for δT and γT (2003–2005) were 45, 41, 12 and 20% for sesame accessions. These values indicate greater variability for αT and sesamin than for the other parameters in all three years. This confirms that αT and sesamin were more variable in sesame accessions; however δT was almost as variable as sesamin. Only δT was significantly correlated with γT ($r^2 = 0.5701$) as depicted in Table 7. These results show that these sesame accessions are genetically variable

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Table 6 Eigenvectors, principal components, coefficients of variation, and standard errors for four chemical traits in sesame seed accessions (2003–2005)

		Principle components					
Analyte	Year	1	2	3	4	Coefficient of variation	SE[a]
Sesamin	2003/2004	0.13	0.83	−0.36	0.37	46%	0.531
	2005	−0.33	0.74	−0.46	0.32	45%	0.315
αT	2003/2004	0.29	0.37	0.87	−0.07	90%	0.028
	2005	−0.41	0.35	0.83	−0.05	91%	0.013
δT	2003/2004	0.68	0.04	−0.30	−0.65	45%	0.252
	2005	0.55	0.53	0.003	−0.63	41%	0.123
γT	2003/2004	0.64	−0.39	0.01	0.65	12%	2.996
	2005	0.63	0.16	0.28	0.69	20%	2.800

[a]SE: Standard error.

Table 7 Correlation matrix of sesamin, α-tocopherol, δ-tocopherol, and γ-tocopherol in *S. indicum* (2003–2005)

		2005		
2003–2004	Sesamin	αT	δT	γT
Sesamin	N/A	0.2080	–0.0045	–0.2866
αT	0.1098	N/A	–0.1999	–0.2294
δT	0.2085	0.1098	N/A	0.5701[a]
γT	−0.1448	0.1251	0.5328	N/A

[a]Correlations significant ($p < 0.001$). N/A: not applicable.

for sesamin, as well as αT, δT and γT. Even though this study was conducted in one environment, we observed that each chemical component was variable among sesame accessions. However, additional research investigating genetic variability among sesame seed accessions implementing different environments is warranted.

CONCLUSION

Genetic, geographical, as well as environmental conditions including soil, irrigation and phenological aspects contribute to variable amounts of sesamin, αT, δT and γT in *S. indicum* plant genotypes. The goal of this investigational study was to devise an HPLC-PAD-ECD system for the detection, characterisation and quantification of sesamin, αT, δT and γT. This is the first report using these techniques and methodologies for evaluating sesamin and desmethyl tocopherol levels in 11 different genotypic accessions from sesame seeds of worldwide origin as well as in the USDA, ARS and PGRCU repository. However, additional sesame seed accessions from other worldwide regions grown in multiple environments are needed to adequately determine possible candidates for use in a breeding programme for the enhancement of sesamin and tocopherol levels in diverse *S. indicum* plant genotypes. The results from these studies may help to reach more definitive conclusions regarding the differences of sesamin and desmethyl tocopherol vitamers in health maintenance and disease prevention based on their use for nutraceutical and pharmacological antioxidant properties.

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